EFFECT OF QUINIDINE, PROCAINE AMIDE AND LIDOCAINE ON CALCIUM TRANSPORT BY SUBCELLULAR PARTICLES OF THE RABBIT HEART

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ABSTRACT

Quinidine has been reported to depress calcium uptake and calcium stimulated ATPase of the dog heart sarcoplasmic reticulum, however, no information concerning the action of this agent on other organelles such as mitochondria and sarcolemma, which are also considered to participate in calcium regulation in heart, is available in the literature. The present investigation was undertaken to study the effects of quinidine on calcium binding and uptake by rabbit heart sarcoplasmic reticulum as well as mitochondria. The action of this agent on Mg^{++}- ATP independent calcium adsorption by these organelles and sarcolemma was also studied. Two other agents, procaine amide and lidocaine were used for comparison purposes.

Quinidine was found to stimulate calcium binding by heavy microsomes (in the presence of ATP) at initial intervals of incubation and at low concentrations of Mg^{++}. Procaine amide, but not lidocaine, also showed stimulation of calcium binding by microsomes. On the other hand, quinidine was found to depress calcium binding by mitochondria. This depressant effect was not apparent at initial intervals of incubation, low concentrations of ATP or Mg^{++}. Lidocaine but not procaine amide also depressed calcium binding by mitochondria at late time intervals of incubation.

Quinidine in concentrations of 10^{-4} and 10^{-3} M produced a marked depressant effect on microsomal (in the presence of ATP and oxalate) and mitochondrial (in the presence of ATP, Pi and succinate) calcium uptake. The depressant effect of quinidine on microsomal and mitochondrial calcium uptake was observed at all concentrations of ATP but was not apparent when low concentrations of Mg^{++} were used in the incubation medium. Procaine amide had no effect on microsomal or mitochondrial calcium uptake whereas lidocaine showed a stimulatory effect only on

microsomal calcium uptake at initial intervals of incubation.

Quinidine at concentrations of 10^{-4} to 10^{-2} M stimulated calcium adsorption (Mg^{++} - ATP independent) by microsomal, mitochondrial and sarcolemmal fractions. Procaine amide had no effect on sarcolemma, stimulated microsomal calcium adsorption and produced a biphasic effect on mitochondrial calcium adsorption. Lidocaine, on the other hand, had a depressant effect on these fractions at high concentrations.

These results suggest that quinidine may be acting at microsomal, mitochondrial and sarcolemma levels. The action of quinidine in terms of calcium adsorption, binding and uptake seems to be of a complex nature and possesses some similarities and differences from other agents, procaine amide and lidocaine.

INTRODUCTION AND STATEMENT OF THE PROBLEM

Quinidine in low concentrations has an antiarrhythmic action, whereas in high concentrations it is known to depress myocardial contractility and produce contracture of skeletal muscle (1 - 3). Various investigators have attempted to explain these pharmacological effects of quinidine on cardiac and skeletal muscles on the basis of its action on calcium transport by sarcoplasmic reticulum (4 - 8). It should be noted that calcium is generally believed to serve as a link between excitation and contraction of the cardiac and skeletal muscles (1, 9 - 12). However, unlike skeletal muscle where sarcoplasmic reticulum plays a predominant role in the regulation of intracellular calcium, the calcium movements in cardiac muscle are considered to be regulated by sarcoplasmic reticulum, mitochondria and sarcolemma (13 - 21). Therefore, it was the purpose of this study to investigate the abilities of both sarcoplasmic reticulum (heavy microsomes), and mitochondria to bind and accumulate calcium in the absence and presence of quinidine under various experimental conditions. In addition, the effects of this agent on the Mg^{++} - ATP independent calcium adsorption by these subcellular fractions and sarcolemma were investigated. Preliminary results reported in this study were presented before the Canadian Federation of Biological Societies (22).

REVIEW OF LITERATURE

A. Current Concept of Regulation of Calcium in the Heart

Calcium is now generally believed to be the final activator of the contractile system of cardiac muscle (1, 10, 12, 20, 21, 23, 24). Current theory states that extracellular calcium enters the heart cell during depolarization and is also released from superficial sites on the sarcolemma. In addition, calcium is considered to be released from internal storage sites such as sarcoplasmic reticulum. The intracellular level of ionized calcium is thus raised above 10^{-7} M. This calcium binds to troponin, the calcium receptive protein at the contractile apparatus and stimulates actomyosin ATPase. Binding of calcium to troponin releases its inhibition upon the actin and myosin system whereas stimulation of ATPase and subsequent hydrolysis of ATP provides the energy for sliding of the myofilaments. The intracellular level of calcium is then lowered to approximately 10^{-7} M by the sarcoplasmic reticulum as well as active efflux of calcium across the sarcolemma thus resulting in relaxation of the cardiac muscle. Although mitochondria are abundant in the cardiac cell and are able to transport calcium, their role in raising and lowering the intracellular concentration of ionized calcium during contraction and relaxation, respectively, has not yet been clearly defined.

While calcium transport properties of sarcoplasmic reticulum and mitochondria have been extensively studied, the sarcolemma of cardiac muscle has not received much attention. This is partly due to the fact that there are difficulties in isolating this fraction in a relatively pure form. However, calcium has been shown to be required for electrical activity of the sarcolemma and its influx has been shown to

contribute, alongwith Na^{+} and K^{+}, to the magnitude and duration of the action potential (20, 21). Recently a number of investigators have demonstrated the presence of a Ca^{++}- stimulated ATPase in heart sarcolemma (25 - 27). This enzyme can be considered to be involved in the movement of calcium across the sarcolemma. Although some of the investigators have reported the isolation of heart sarcolemma with active Na^{+}- K^{+} ATPase and adenylate cyclase (28 - 30), no information concerning its ability to transport calcium is available.

The basement membrane that coats the heart sarcolemma is a mucopolysaccharide or mucopolysaccharide - protein complex containing negatively charged sites (31). This basement membrane also lines the surface of the T-system. It has been demonstrated by Scott (32) that the mucopolysaccharides have a strong affinity for cations notably calcium. Recently, Madeira et al. (33) were able to show that sarcolemmal vesicles isolated from skeletal muscle have the ability to bind ions notably Ca^{++}, Mg^{++} and Zn^{++}. These workers have proposed that anionic groups in the sarcolemma, either phosphoric sites of phospholipids or carboxyl groups of proteins may bind these cations. If the phospholipids were removed by phospholipase C, there was a decrease in cations binding. Zinc ions showed the highest affinity, whereas Ca^{++} and Mg^{++} had the same affinity. Cardiac sarcolemma can also be conceived to have similar calcium binding sites which may serve as a source of trigger calcium in the process of excitation-contraction coupling (34, 35).

Since the work of Ebashi and Lipmann (36), in which they were able to show the ability of fragmented sarcoplasmic reticulum vesicles to accumulate radio-

active calcium in the presence of ATP and Mg^{++}, it is generally accepted that calcium available for binding to troponin is derived almost entirely from the sarcoplasmic reticulum. This calcium is made available by passive release from the sarcoplasmic reticulum after the arrival of the wave of depolarization from the T-system (12).

The role of sarcoplasmic reticulum in the control of calcium levels in the muscle cell is also indicated by the very close anatomical relationship of the sarcoplasmic reticulum and myofibrils (37, 38). Furthermore, in vitro studies have shown that heart, as well as skeletal muscle sarcoplasmic reticulum can bind and accumulate calcium (17, 18, 39 - 41). In the presence of calcium precipitating agents such as oxalate or inorganic phosphate, cardiac sarcoplasmic reticulum has been shown to accumulate micromolar quantities of calcium. Ikemoto et al. (42) have shown the presence of insoluble calcium salts within the microsomal vesicles. In the absence of the precipitating agent, the intravesicular precipitation of calcium salts does not occur. The term "calcium binding" is commonly applied to the ability of microsomes to take up calcium in the absence of the precipitating agents. On the other hand, the term "calcium uptake" is usually employed to signify calcium accumulation by microsomes in the presence of the precipitating agents.

A number of experimental conditions have been shown to influence calcium transport by cardiac sarcoplasmic reticulum. The concentrations of Na^{+} and K^{+} have been found to affect calcium uptake (43, 44). High concentrations of NaCl and KCl were demonstrated to inhibit calcium uptake, whereas low concentrations of these ions caused an enhancement of initial rate and maximal uptake; K^{+} was more effective than Na^{+}. Both ATP and Mg^{++} were also found to be important for calcium uptake, which

is now well established to be an energy dependent process. When ATP and Mg^{++} were omitted from the incubation medium only a small amount of calcium was accumulated by the fragments of sarcoplasmic reticulum and this process has been referred to as passive calcium adsorption which is primarily due to a physical interaction of calcium with the membrane (40). Lee (45) has demonstrated that isolated sarcoplasmic reticulum could release calcium in vitro following electrical stimulation. This calcium release was found to be dependent upon the frequency of stimulation.

The controversy of whether sarcoplasmic reticulum plays the predominant role as a storage site for calcium in the heart has been raised by morphological and biochemical evidence. It should be recognized that most of the work concerning the mechanisms of excitation-contraction coupling has been done using sarcoplasmic reticulum from skeletal muscle. Although the properties of sarcoplasmic reticulum from heart has been shown to be similar to that of skeletal muscle, cardiac muscle has a sparse sarcotubular system (38). Furthermore, the absence of transverse tubules and the paucity of sarcoplasmic reticulum in the frog ventricle suggest the existence of other mechanisms for the regulation of intracellular calcium in cardiac contraction (46). Also kinetic evidence provided by Katz and Repke (47) has shown that calcium uptake by cardiac sarcoplasmic reticulum was too slow to account for relaxation.

From the original work of Cleland and Slater (48), in which they showed that mitochondria could take up calcium, attention is being given to this event occuring in heart mitochondria as a possible mechanism for the regulation of intracellular calcium. Various workers (44, 49 - 52) have shown that mitochondria in the presence of ATP can accumulate Ca^{++}. When mitochondria were equilibrated with inorganic phosphate

and calcium, electron dense granules, possibly due to the precipitation of calcium salts, were observed under the electron microscope (50). The hydrolysis of ATP by mitochondria has been shown to occur concommittantly with calcium accumulation as well as H^+ ejection into the incubation medium (52). The energy dependent calcium uptake by mitochondria is inhibited by oligomycin (53) and azide (44). Divalent cations such as Mg^{++} and Mn^{++} stimulate calcium uptake by mitochondria whereas ADP, orthophosphate and high concentrations of Na^+ and K^+ reduce calcium accumulation (51).

That mitochondria do play a role in heart calcium metabolism has been supported by the work of Patriarca and Carafoli (13) in which these workers have shown that most of the injected $^{45}Ca^{++}$ in animals was accumulated by heart mitochondria and that the specific activity of calcium in mitochondria was higher than that of the sarcoplasmic reticulum. Similar results were found by Dhalla et al. (18) in the isolated perfused heart preparations. Also mitochondria have been found to inhibit super-precipitation by myofibrils (54). Mitochondria are abundant in heart cells and are situated in close proximity to the myofibrils (14, 16, 17). Furthermore, the hearts were unable to relax when perfused with oligomycin, a well known inhibitor of calcium uptake by mitochondria (16). These studies tend to support the role of mitochondria in the regulation of intracellular calcium in the heart. Haugaard and his associates (15) have also implicated this role of mitochondria in the contraction-relaxation cycle of the myocardium.

B. Effects of Various Pharmacologic Agents on Calcium Transport in Heart

A number of pharmacologically active agents have been shown to influence the calcium transporting system of both sarcoplasmic reticulum and mitochondria of the heart. Conflicting reports in the literature have not settled the mode of action of the cardiac glycosides. Many investigators have reported that ouabain and strophanthidin depress calcium uptake in mitochondria and sarcoplasmic reticulum (55 - 58). However, a number of other investigators claim that the cardiac glycosides have no effect on calcium accumulation by the subcellular particles (17, 47, 59, 60). In view of these results, it is difficult to explain the positive inotropic effects of the cardiac glycosides on the basis of their action on the calcium transport by the subcellular particles.

The effect of the catecholamines and cyclic 3',5'-adenosine monophosphate (cyclic AMP) on calcium transport has also received much attention in order to explain the positive inotropic actions of these agents. However, conflicting reports have appeared in the literature. Some investigators have reported that epinephrine, norepinephrine and cyclic AMP augment calcium accumulation by sarcoplasmic reticulum (61 - 64). Sarcoplasmic reticulum has also been shown to contain adenylate cyclase (65 - 67). The stimulation of this enzyme by catecholamines with increased formation of cyclic AMP is considered to account for the action of catecholamines (63). Thus the increased calcium content of the sarcoplasmic reticulum allows more calcium to be made available to the contractile apparatus during depolarization (63). The hypothesis that increased levels of cyclic AMP cause an augmentation of the sarcotubular calcium pool is interesting in the sense that it helps to explain the positive inotropic

effect of various interventions at the molecular level. However, other workers have failed to confirm the existence of such an effect of cyclic AMP (47, 68). Likewise, both glucagon and catecholamines have been reported to be ineffective in enhancing calcium uptake by the heart sarcotubular vesicles (47, 59, 68). These studies suggest that the proposed mechanism of hormonal action on calcium transport across sarcotubular vesicles should be taken with some caution.

Recently cardiac sarcotubular membranes possessing adenylate cyclase and calcium accumulating activities have been shown to contain cyclic AMP stimulated protein kinase (69). It has been suggested that cyclic AMP stimulates the formation of a membrane phosphoprotein which mediates the cyclic AMP induced changes in calcium transport. The experiments described by Kirchberger et al. (70) revealed that the addition of exogenous protein kinase in high concentrations was necessary to show the effect of cyclic AMP on calcium uptake. Since these workers failed to observe an action of cyclic AMP - protein kinase on calcium binding by sarcotubular vesicles, the significance of their reported effect on calcium uptake is subject to some serious questions. In this regard, it should be mentioned that Gertz et al. (71) have attributed the stimulatory effect of cyclic AMP to a non-specific protective effect of the nucleotide to retard the in vitro deterioration of cardiac sarcotubular calcium uptake. Furthermore, cyclic AMP has been shown to be without an effect on the phosphoryl transfer reaction, which may represent the formation of a carrier system and thus facilitate the influx of calcium into the sarcotubular vesicles (72).

The barbiturates have been found to depress calcium uptake by sarcoplasmic reticulum and mitochondria. Nayler et al. (73) and Lain et al. (74) have reported

that millimolar concentrations of sodium pentobarbital interfered with the ability of cardiac microsomes to accumulate calcium. Briggs et al. (75) found that amytal inhibited calcium uptake in microsomal vesicles of the dog heart but the inhibition could be prevented by strophanthin. However, Dransfield et al. (76) failed to detect any effect of 1 to 6 mM pentobarbital on calcium uptake by microsomes but have reported inhibition of calcium uptake by mitochondria.

The beta-receptor blocking agents have been found to depress myocardial contractility and this action has been attributed to the effects of these agents on the calcium transporting system of the heart. Various investigators (6, 77 - 79) have shown that propranolol, alprenolol and pronethalol are capable of depressing calcium uptake by heart mitochondria as well as sarcoplasmic reticulum. The results of these experiments are difficult to interpret because of the high concentrations of drug used as compared to that in vivo.

The effects of the antiarrhythmic drugs on heart function have usually been interpreted on the basis that these agents are able to alter the electrophysiological properties of the myocardium (2, 3, 80). However, a number of reports have appeared recently in the literature with respect to the effects of these agents on calcium transport in heart and skeletal muscle subcellular systems. In both cases, quinidine at concentrations of 10^{-4} and 10^{-3} M has been shown to depress calcium uptake and Ca^{++}- activated ATPase of sarcoplasmic reticulum (4 - 7). These concentrations of quinidine cause twitch potentiation and contracture in skeletal muscle (81, 82) whereas cardiac muscle in the presence of this agent shows a significant depression of tension development (80). Isaacson and Sandow (82) attributed the effect of quinidine on skeletal

muscle such that this agent is able to either depress calcium accumulation by sarcoplasmic reticulum or release calcium from this organelle thereby raising the free myoplasmic concentration of this ion and prolonging the active state. However, Fuchs et al. (4) attributed the reduction of myocardial tension development to depression of calcium accumulation by heart sarcoplasmic reticulum but the increase in myoplasmic calcium would be quickly reduced by other calcium transport systems such as mitochondria and sarcolemma. Some impetus was given to this concept by Carvalho (5) when he showed that skeletal muscle sarcoplasmic reticulum in the presence of the optical isomer of quinidine, quinine, was able to release the passively bound calcium, that is, in the absence of ATP and depress the selective binding of calcium induced by ATP. Although quinidine depressed calcium uptake in vitro, this is not definitive evidence for the antiarrhythmic effect of the drug in vivo. This is partly due to the fact that the concentrations used are greater than the therapeutic range. However, Conn (84) has shown that quinidine binds to sarcolemma and mitochondria and Balzer (7) using tritium labelled dihydroquinidine, has shown binding of an amount of quinidine to skeletal muscle sarcoplasmic reticulum comparable to the concentration required to elicit 50% inhibition of calcium uptake.

Several reports have appeared in the literature that are contradictory with observations of other workers. The work of Scales and McIntosh (85) has shown that quinidine at a concentration of 2×10^{-4} M caused a small but significant increase in the total ATP dependent calcium binding and uptake by skeletal muscle sarcoplasmic reticulum. These workers have also reported that quinidine stimulated total ATPase activity of this fraction. These results are in disagreement with Fuchs et al. (4)

and Balzer (7) who have reported a depression of the ATPase activity of sarcoplasmic reticulum from both heart and skeletal muscle. Quinidine has also been shown to act on calcium transporting systems other than sarcoplasmic reticulum. Noack (77) has shown that quinidine decreased the velocity of calcium uptake and state 4 respiration by heart mitochondria without influencing the efficiency of oxidative phosphorylation. Graca and Van Zwieten (86) have shown that 5×10^{-5} M quinidine reduced the rate of cellular and extracellular $^{45}Ca^{++}$ exchange in electrically driven isolated guinea pig atria. They attribute their results to a diminished membrane permeability for calcium during excitation due to quinidine. Madeira and Carvalho (33) working with skeletal muscle sarcolemma have shown that quinine competitively inhibited calcium binding in a medium containing approximately 100 to 200 μM calcium. Quinidine (1 mM) also has been shown to reduce Na^{+}- K^{+} ATPase activity in bovine heart sarcolemma by 50% (87); this inhibition was not Mg^{++} dependent because various concentrations of Mg^{++} (3 to 4 mM) showed no significant change in the inhibitory effect. These results showing the effect of quinidine on Na^{+}- K^{+} ATPase can be interpreted to reflect the action of the drug at sarcolemma.

METHODS

A. Isolation of Sarcoplasmic Reticulum and Mitochondria

Healthy male albino rabbits were sacrificed by cervical dislocation and the hearts quickly excised and placed in ice cold homogenizing medium containing 0.25 M sucrose, 1 mM EDTA and 20 mM Tris-HCl, pH 7.0. The atria were dissected out and the ventricles trimmed of fat and connective tissue. The ventricles were weighed and minced with scissors. The tissue was homogenized in 10 volumes of media in a Waring Blendor for 2 x 20 seconds with a 1 minute interval. The homogenate was filtered through 2 layers of gauze and centrifuged at 1,000 x g for 20 minutes to remove cell debris. The supernatant was centrifuged at 10,000 x g for 20 minutes to obtain the mitochondrial pellet. This pellet was washed and suspended in the homogenizing medium, spun at 1,000 x g for 10 minutes, the residue discarded and the supernatant further centrifuged at 8,000 x g for 10 minutes to obtain mitochondrial fraction. The post 10,000 x g supernatant was centrifuged at 40,000 x g for 45 minutes, the pellet washed, resuspended in 0.6 M KCl containing 20 mM Tris-HCl, pH 6.8 - 7.0 and centrifuged at 40,000 x g for 45 minutes to separate heavy microsomes. Both mitochondrial and microsomal fractions were suspended in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.0 at a protein concentration of 1 to 2 mg/ml. This procedure for isolating mitochondria and heavy microsomes is similar to that used previously in this laboratory (88).

B. Isolation of Sarcolemma Fraction

Rabbit heart ventricles were washed thoroughly, diced with a pair of scissors and homogenized in 10 volumes of 50 mM Tris-HCl, pH 7.4, containing

1 mM EDTA in a Waring Blendor for 2 x 30 seconds with an interval of 1 minute. The homogenate was filtered through gauze and centrifuged at 1,000 x g for 10 minutes. The sediment was suspended in 25 volumes of 10 mM Tris-HCl, pH 7.4 and stirred in the cold room for 30 minutes and centrifuged at 1,000 x g for 10 minutes. The residue was then suspended in 25 volumes of 10 mM Tris-HCl, pH 8.0, stirred for 30 minutes and centrifuged at 1,000 x g for 10 minutes. The sediment was suspended in 10 mM Tris-HCl, pH 7.4, stirred for 30 minutes and centrifuged at 1,000 x g for 10 minutes; this step was repeated again. The sediment was suspended in 25 volumes of 10 mM Tris-HCl, pH 7.4, extracted with 0.4 M LiBr for 45 minutes and centrifuged at 1,000 x g for 10 minutes. This sediment was washed with 10 mM Tris-HCl, pH 7.4, and suspended in 1 mM Tris-HCl, pH 7.4, and immediately used. The fraction isolated in the above manner will be referred to as sarcolemmal fraction. This method is the same as that described by McNamara (89) and is a modification of that described by Kono and Colowich (90).

C. Measurement of Calcium Transport

1. Calcium binding

Calcium binding by mitochondria, microsomes and sarcolemma was measured in a medium consisting of 100 mM KCl, 2 mM $MgCl_2$, 20 mM Tris-HCl, pH 6.8 to 7.0, 0.1 mM $^{45}CaCl_2$, 2 mM Na_2- ATP in a total volume of 1 to 3 ml. The mitochondrial protein concentration in the incubation medium was 0.2 to 0.3 mg/ml. The sarcolemmal and microsomal fractions were incubated at a protein concentration

of 0.1 to 0.2 mg/ml. The fractions were pre-incubated for 3 minutes at 25°C in the presence of ATP and drug. The reaction was started by the addition of $^{45}CaCl_2$ (New England Nuclear, Dorval, Quebec) and stopped by millipore filtration (Millipore Corporation, pore size 0.45 μ). The amount of ^{45}Ca in 0.1 ml of filtrate was analyzed in 10 ml of Bray's solution in a Packard Tri-Carb scintillation spectrometer.

2. Calcium uptake by microsomes

Calcium uptake by microsomes was measured by the method described for binding except that 5 mM potassium oxalate was added to the incubation medium and 0.02 to 0.05 mg/ml microsomal protein at a temperature of 37°C was employed.

3. Calcium uptake by mitochondria

Calcium uptake by mitochondria was determined at 37°C in the calcium binding medium in the presence of 4 mM inorganic phosphate (Pi) and 5 mM succinate. A mitochondrial protein concentration of 0.1 to 0.2 mg/ml was used.

4. Calcium adsorption

Calcium adsorption by microsomal, mitochondrial, and sarcolemmal fractions was studied by the method described for calcium binding except that Mg^{++} and ATP were excluded from the incubation medium. The protein concentration in the incubation medium was 0.1 to 0.2 mg/ml. The temperature of the medium was 25°C.

All of the above procedures for isolating subcellular fractions were carried out in a cold room at 0 to 4°C. Either a Sorvall RC2-B or an International B20-A refrigerated centrifuge was used. The protein concentration was determined according

to the method of Lowry et al. (91). The results were analyzed statistically by the conventional student "t" test.

Quinidine gluconate U.S.P. was obtained from Eli Lilly and Company, Indianapolis, Indiana, procaine amide hydrochloride, U.S.P. (Pronestyl) from E.R. Squibb and Sons Ltd., Montreal, Quebec and lidocaine hydrochloride, U.S.P. (Xylocaine) from Astra Chemicals Ltd., Mississauga, Ontario.

RESULTS

A. Calcium Binding

Calcium binding by heart heavy microsomes and mitochondria was determined in the presence of various concentrations of quinidine and the results are described in Figure 1. No significant ($P > 0.05$) changes in calcium binding by heavy microsomes was observed. However, mitochondrial calcium binding was significantly ($P < 0.05$) depressed by 10^{-4} and 10^{-3} M quinidine. The time course of calcium binding by heart heavy microsomes in the presence of 1 mM quinidine, procaine amide and lidocaine is shown in Table 1. Quinidine and procaine amide had a significant ($P < 0.05$) stimulatory action at the earlier time intervals of incubation. On the other hand, lidocaine exerted no significant ($P > 0.05$) effect throughout the course of incubation. The time course of calcium binding by heart mitochondria in the presence of 1 mM quinidine, procaine amide and lidocaine is shown in Table 2. Quinidine and lidocaine, unlike procaine amide, showed a significant ($P < 0.05$) depression of calcium binding at the later time intervals of incubation.

Calcium binding by heart heavy microsomes and mitochondria in the absence and presence of 1 mM quinidine was determined at various concentrations of Mg^{++} in the incubation medium. The results concerning the effect of quinidine are shown in Figure 2. At low concentrations of Mg^{++} quinidine exerted a significant ($P < 0.05$) stimulatory effect on microsomal calcium binding. On the other hand, quinidine did not influence mitochondrial calcium binding at concentrations of Mg^{++} up to 1.5 mM; a significant ($P < 0.05$) decrease in calcium binding was apparent at 2 mM Mg^{++}. Calcium binding by heart heavy microsomes and

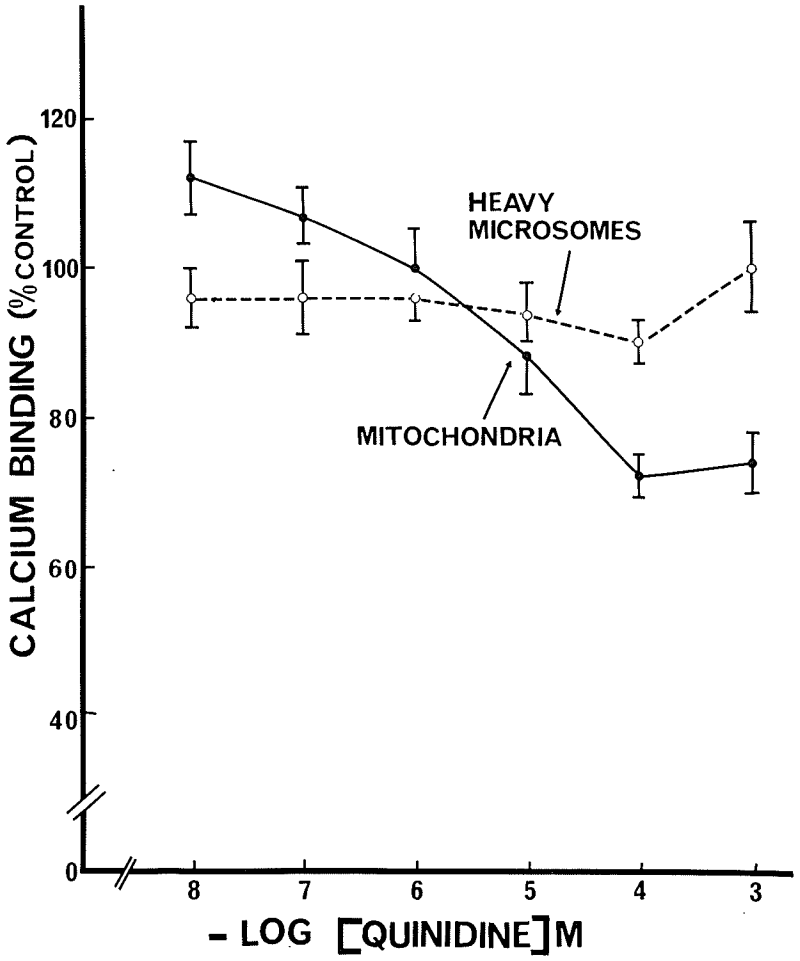

FIGURE 1 Effect of various concentrations of quinidine on calcium binding by rabbit heart heavy microsomes and mitochondria. The incubation medium was the same as that described for calcium binding in the Methods section except that 10 mM Mg^{++} and 4 mM ATP were used. The time of incubation of the subcellular fractions with $^{45}Ca^{++}$ was 5 minutes. The control values for microsomal and mitochondrial calcium binding were 49 $\pm$ 6 and 43 $\pm$ 3 nmoles/mg protein respectively. Each value is a mean $\pm$ S.E. of 5 experiments.

TABLE 1

Time Course of Calcium Binding by Rabbit Heart Heavy Microsomes in the Presence of 1 mM Quinidine, Procaine Amide and Lidocaine

Incubation time	Heavy microsomal calcium (nmoles/mg protein)**			
	Control	Quinidine	Procaine amide	Lidocaine
30 sec	37 ± 3	61 ± 3*	45 ± 3	41 ± 2
1 min	47 ± 3	64 ± 1*	66 ± 4*	54 ± 3
2 min	55 ± 2	63 ± 3	66 ± 3*	63 ± 3
5 min	60 ± 3	71 ± 4	76 ± 2*	72 ± 4
10 min	72 ± 2	72 ± 4	74 ± 4	75 ± 3

* $P < 0.05$

** Each value is a mean ± S.E. of 6 experiments. The incubation medium was the same as that described for calcium binding in the Methods section.

TABLE 2

Time Course of Calcium Binding by Rabbit Heart Mitochondria in the Presence of 1 mM Quinidine, Procaine Amide and Lidocaine

	Mitochondrial calcium (nmoles/mg protein)**			
Incubation time	Control	Quinidine	Procaine amide	Lidocaine
30 sec	39 ± 3	47 ± 4	52 ± 4	36 ± 3
1 min	45 ± 3	46 ± 3	56 ± 4	55 ± 2
2 min	64 ± 2	48 ± 2*	59 ± 3	53 ± 3*
5 min	69 ± 4	54 ± 3*	76 ± 4	53 ± 2*
10 min	73 ± 3	58 ± 4*	79 ± 4	54 ± 4*

* $P < 0.05$

** Each value is a mean ± S.E. of 6 experiments. The incubation medium was the same as that described for calcium binding in the Methods section.

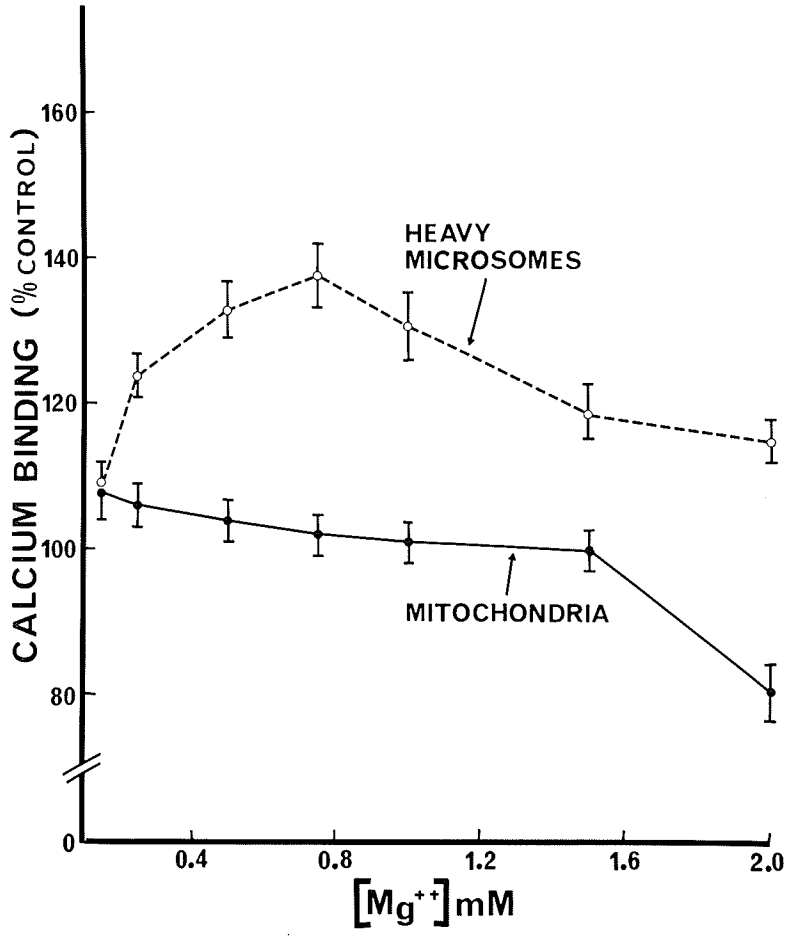

FIGURE 2 Influence of Mg^{++} on the effect of 1 mM quinidine on calcium binding by rabbit heart heavy microsomes and mitochondria. The incubation medium was the same as that described in the Methods section except various concentrations of Mg^{++} were employed. The time of incubation of the subcellular fractions with $^{45}Ca^{++}$ was 5 minutes. Each value is a mean $\pm$ S.E. of 5 experiments.

mitochondria at various concentrations of ATP in the absence and presence of 1 mM quinidine is shown in Table 3. Heavy microsomes in the presence of quinidine over the concentration range of ATP tested showed no significant ($P > 0.05$) differences in calcium binding from that of microsomes incubated in the absence of the drug. The depression due to quinidine on calcium binding by mitochondria was not observed at low concentrations of ATP whereas quinidine (1 mM) significantly ($P < 0.05$) depressed mitochondrial calcium binding at higher concentrations (1 to 5 mM) of ATP.

B. Calcium Uptake

The effect of various concentrations of quinidine or calcium uptake by heart heavy microsomes and mitochondria is shown in Figure 3. Quinidine at low concentrations exerted no effect ($P > 0.05$); however, at high concentrations (10^{-4} and 10^{-3} M) quinidine significantly ($P < 0.01$) depressed calcium uptake by both heavy microsomes and mitochondria. The time course of calcium uptake by heavy microsomes in the presence of 1 mM quinidine, procaine amide and lidocaine is shown in Table 4. Significant depressant action ($P < 0.05$) of quinidine became apparent after 2 minutes of incubation. Microsomal calcium uptake in the presence of procaine amide was unaffected ($P > 0.05$) whereas lidocaine significantly ($P < 0.05$) stimulated uptake during the first 2 minutes of incubation.

The time course of calcium uptake by heart mitochondria in the presence of 1 mM quinidine, procaine amide and lidocaine is shown in Table 5. Mitochondrial calcium uptake was significantly ($P < 0.05$) depressed by quinidine during the 10 minute incubation period. Procaine amide and lidocaine exerted no significant

TABLE 3

Influence of ATP on the Effect of 1 mM Quinidine on Calcium Binding by Rabbit Heart Heavy Microsomes and Mitochondria

	Calcium binding (nmoles/mg protein)**			
	Heavy microsomes		Mitochondria	
(ATP) mM	Control	Quinidine	Control	Quinidine
0.05	38 ± 3	30 ± 2	35 ± 2	39 ± 3
0.10	47 ± 2	53 ± 4	40 ± 2	48 ± 2
0.50	53 ± 3	57 ± 4	57 ± 3	53 ± 3
1.00	59 ± 3	55 ± 3	60 ± 3	45 ± 4*
2.00	71 ± 4	65 ± 2	68 ± 4	50 ± 2*
5.00	35 ± 2	31 ± 2	59 ± 2	47 ± 3*

* $P < 0.05$

** Each value is a mean ± S.E. of 5 experiments. The incubation medium was the same as that described for calcium binding in the Methods section except that various concentrations of ATP were employed.

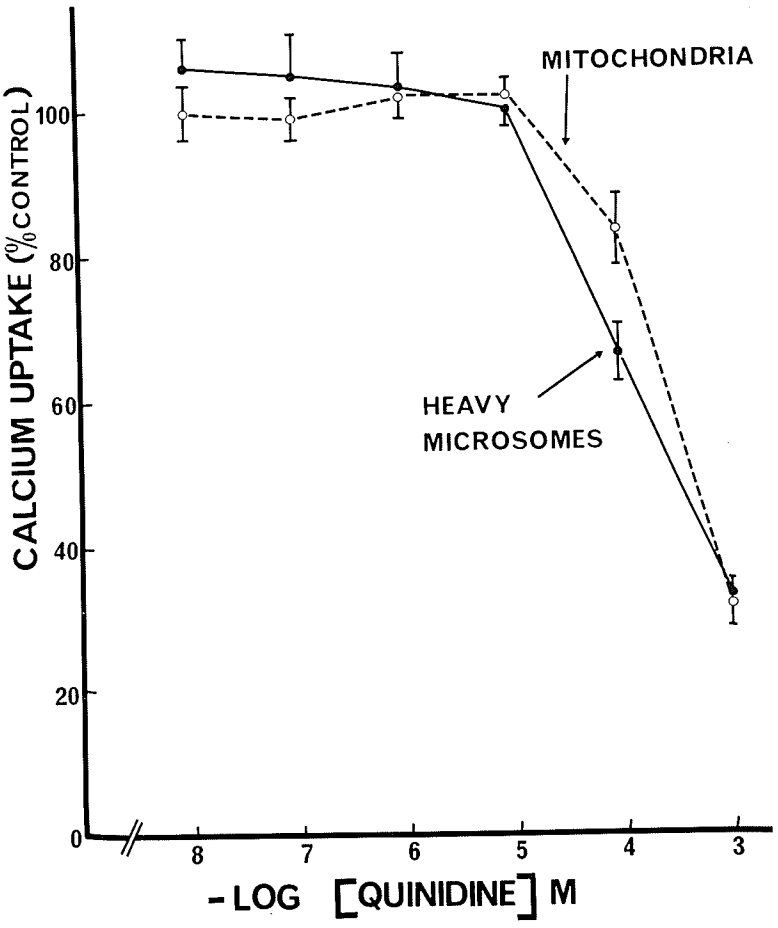

FIGURE 3 Effect of various concentrations of quinidine on calcium uptake by rabbit heart heavy microsomes and mitochondria. The incubation medium was the same as described for calcium uptake in the Methods section except that 10 mM Mg^{++} and 4 mM ATP were used. The time of incubation of the subcellular fractions with $^{45}Ca^{++}$ was 5 minutes. The control values for microsomal and mitochondrial calcium uptake were 1342 $\pm$ 162 and 110 $\pm$ 4 nmoles/mg protein respectively. Each value is a mean $\pm$ S.E. of 5 experiments.

TABLE 4

Time Course of Calcium Uptake by Rabbit Heart Heavy Microsomes in the Presence of 1 mM Quinidine, Procaine Amide and Lidocaine

	Heavy microsomal calcium (nmoles/mg protein)**			
Incubation time	Control	Quinidine	Procaine amide	Lidocaine
30 sec	355 ± 34	457 ± 22	476 ± 35	675 ± 35*
1 min	529 ± 37	490 ± 21	585 ± 38	1068 ± 80*
2 min	813 ± 24	586 ± 42*	718 ± 35	1280 ± 102*
5 min	1272 ± 79	648 ± 53*	1047 ± 70	1385 ± 110
10 min	1291 ± 85	801 ± 45*	1162 ± 104	1414 ± 107

* $P < 0.05$

** Each value is a mean ± S.E. of 6 experiments. The incubation medium was the same as that described for calcium uptake in the Methods section.

TABLE 5

Time Course of Calcium Uptake by Rabbit Heart Mitochondria in the Presence of 1 mM Quinidine, Procaine Amide and Lidocaine

	Mitochondrial calcium (nmoles/mg protein)**			
Incubation time	Control	Quinidine	Procaine amide	Lidocaine
30 sec	128 ± 4	81 ± 3*	138 ± 6	127 ± 6
1 min	138 ± 2	112 ± 6*	140 ± 3	129 ± 3
2 min	155 ± 3	125 ± 4*	143 ± 4	156 ± 4
5 min	169 ± 5	130 ± 5*	153 ± 3	185 ± 7
10 min	203 ± 8	142 ± 6*	179 ± 6	194 ± 7

* $P < 0.05$

** Each value is a mean ± S.E. of 6 experiments. The incubation medium was the same as that described for calcium uptake in the Methods section.

($P > 0.05$) effect on mitochondrial calcium uptake during the course of incubation.

The effect of various concentrations of Mg^{++} on calcium uptake by heart heavy microsomes in the absence and presence of 1 mM quinidine is shown in Figure 4. At Mg^{++} concentrations less than 0.75 mM quinidine exerted no significant ($P > 0.05$) effect on microsomal calcium uptake. At higher concentrations of Mg^{++} quinidine significantly ($P < 0.05$) depressed calcium accumulation by the microsomal fraction. Preliminary results showed a similar trend of quinidine action on mitochondrial calcium uptake. Calcium uptake by heart heavy microsomes and mitochondria in the absence and presence of 1 mM quinidine at various concentrations of ATP is shown in Table 6. Both microsomal and mitochondrial calcium uptake were significantly ($P < 0.05$) reduced in the presence of quinidine at all ATP concentrations tested.

C. Calcium Adsorption

Heavy microsomal calcium adsorption (Mg^{++}- ATP independent binding) in the presence of various concentrations of quinidine, procaine amide and lidocaine is shown in Figure 5. Low concentrations of quinidine (10^{-7} to 10^{-4} M) and procaine amide (10^{-7} to 10^{-3} M) did not influence adsorption. At high concentrations these drugs significantly ($P < 0.05$) stimulated calcium adsorption by heavy microsomes. Lidocaine, on the other hand, showed no effect at low concentrations (10^{-7} to 10^{-3} M) but at higher concentrations it significantly ($P < 0.05$) depressed calcium adsorption.

Mitochondrial calcium adsorption in the presence of various concentrations

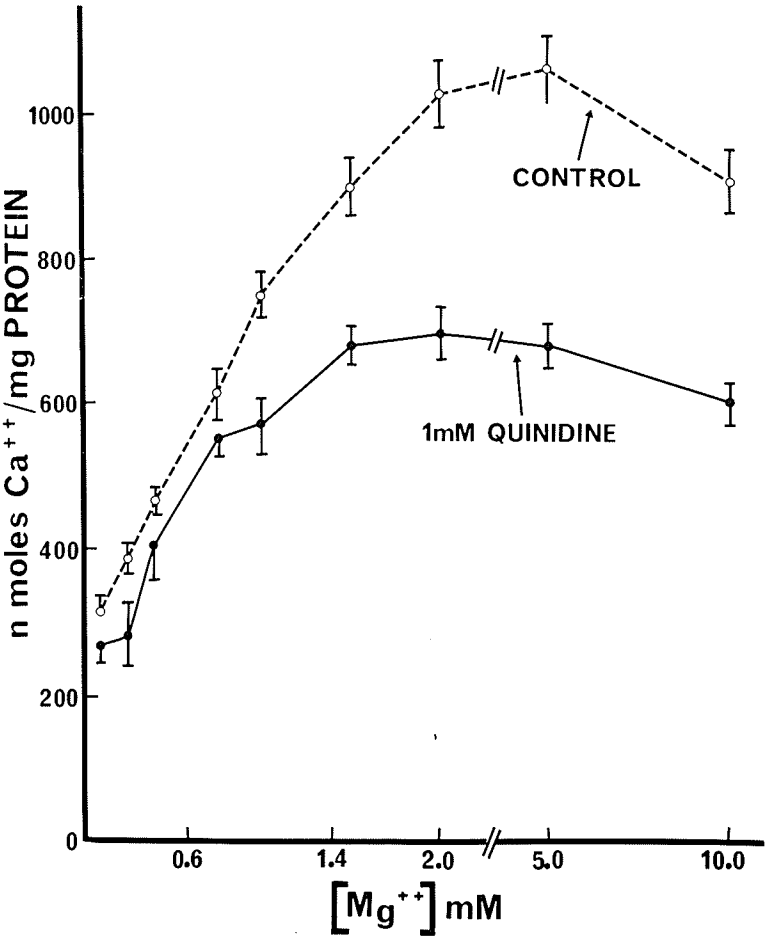

FIGURE 4 Influence of Mg^{++} on the effect of 1 mM quinidine on calcium uptake by rabbit heart heavy microsomes. The incubation medium was the same as that described in the Methods section except various concentrations of Mg^{++} were employed. The time of incubation of the heavy microsomes with $^{45}Ca^{++}$ was 5 minutes. Each value is a mean $\pm$ S.E. of 5 experiments.

TABLE 6

Influence of ATP on the Effect of 1 mM Quinidine on Calcium Uptake by Heart Heavy Microsomes and Mitochondria

	Calcium uptake (nmoles/mg protein)**			
	Heavy microsomes		Mitochondria	
(ATP) mM	Control	Quinidine	Control	Quinidine
0.05	563 ± 25	394 ± 19*	85 ± 5	63 ± 4*
0.10	620 ± 22	433 ± 26*	88 ± 4	69 ± 4*
0.50	844 ± 30	534 ± 20*	101 ± 4	77 ± 5*
1.00	1059 ± 32	739 ± 35*	113 ± 6	87 ± 4*
2.00	1209 ± 42	860 ± 44*	133 ± 5	94 ± 5*
5.00	440 ± 39	322 ± 28*	91 ± 3	67 ± 3*

* $P < 0.05$

** Each value is a mean ± S.E. of 5 experiments. The incubation medium was the same as that described for calcium uptake in the Methods section except that various concentrations of ATP were employed.

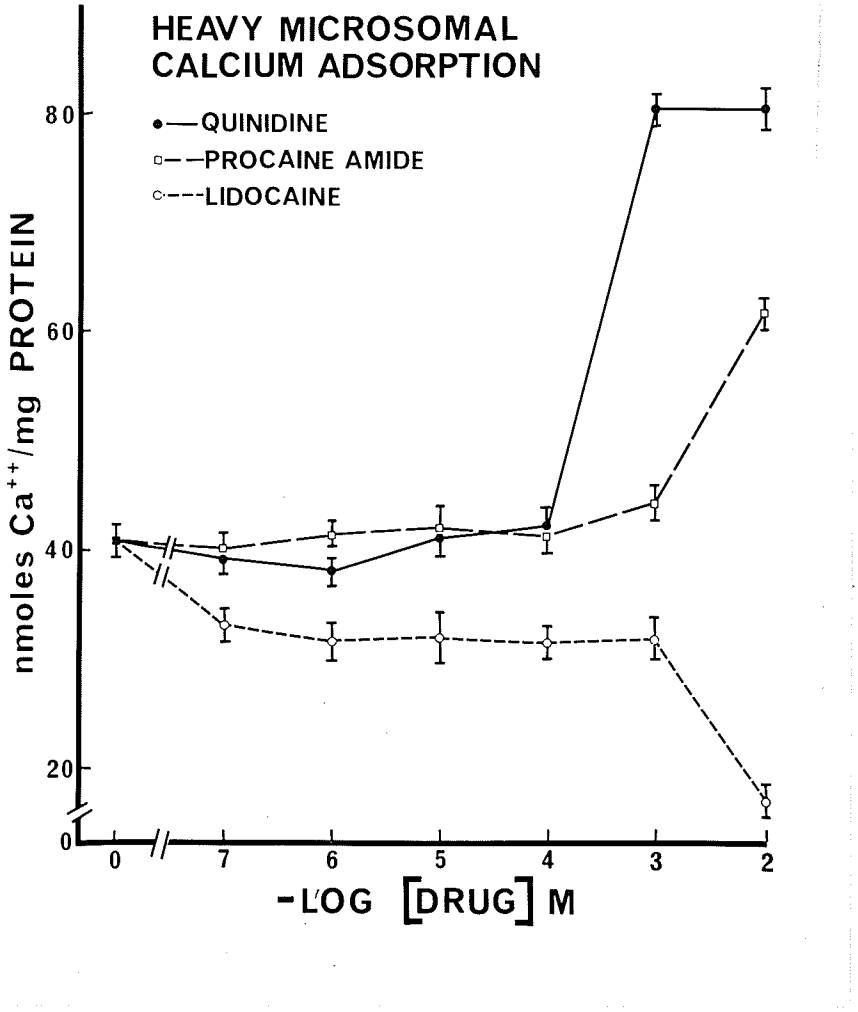

FIGURE 5 Effect of various concentrations of quinidine, procaine amide and lidocaine on calcium adsorption by rabbit heart heavy microsomes. The incubation medium was the same as that described for calcium binding in the Methods section except that Mg^{++} and ATP were excluded from the medium. The time of incubation of the heavy microsomes with $^{45}Ca^{++}$ was 5 minutes. The control values for microsomal calcium adsorption was 42 $\pm$ 3 nmoles/mg protein. Each value is a mean $\pm$ S.E. of 4 experiments.

of quinidine, procaine amide and lidocaine is shown in Figure 6. Quinidine at concentrations greater than 10^{-4} M markedly ($P < 0.01$) stimulated calcium adsorption. Procaine amide had a slight but significant ($P < 0.05$) depressant effect at concentrations less than 10^{-5} M but showed a significant ($P < 0.05$) stimulation at a concentration of 10^{-2} M. On the other hand, lidocaine produced a slight but significant ($P < 0.05$) depressant effect at high concentrations (10^{-4} to 10^{-2} M).

Sarcolemmal calcium adsorption in the presence of various concentrations of quinidine, procaine amide and lidocaine is shown in Figure 7. Quinidine significantly ($P < 0.05$) enhanced calcium adsorption but only at high concentrations (10^{-3} and 10^{-2} M) whereas procaine amide exerted no effect over the range of concentrations tested. Lidocaine, on the other hand, significantly ($P < 0.05$) depressed calcium adsorption at a high concentration (10^{-2} M).

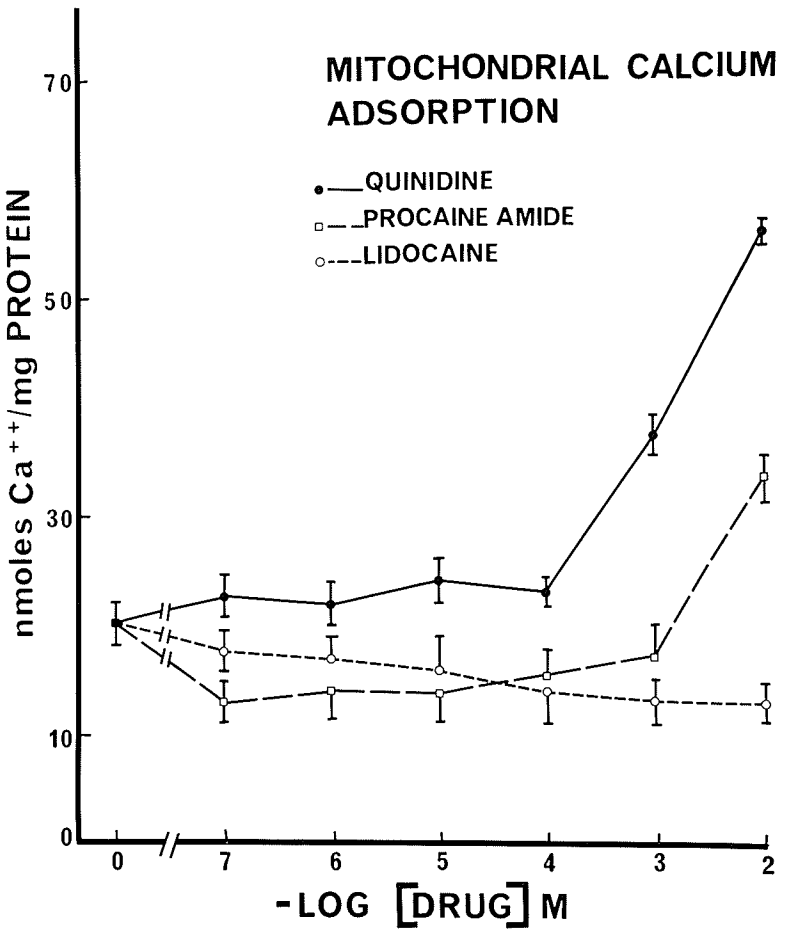

FIGURE 6 Effect of various concentrations of quinidine, procaine amide and lidocaine on calcium adsorption by rabbit heart mitochondria. The incubation medium was the same as that described for calcium binding in the Methods section except that Mg^{++} and ATP were excluded from the medium. The time of incubation of the heavy microsomes with $^{45}Ca^{++}$ was 5 minutes. The control values for mitochondrial calcium adsorption was 21 $\pm$ 2 nmoles/mg protein. Each value is a mean $\pm$ S.E. of 4 experiments.

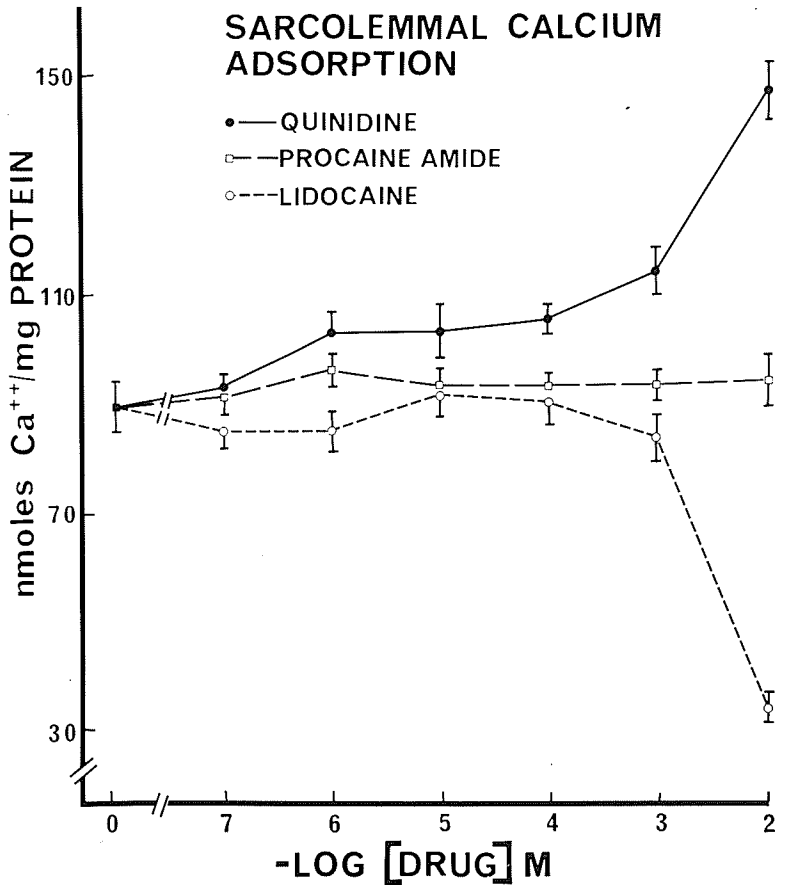

FIGURE 7 Effect of various concentrations of quinidine, procaine amide and lidocaine on calcium adsorption by rabbit heart sarcolemma. The incubation medium was the same as that described for calcium binding in the Methods section except that Mg^{++} and ATP were excluded from the medium. The time of incubation of the heavy microsomes with $^{45}Ca^{++}$ was 5 minutes. The control value for sarcolemmal calcium adsorption was 90 $\pm$ 5 nmoles/mg protein. Each value is a mean $\pm$ S.E. of 4 experiments.

DISCUSSION

The calcium transport system by subcellular particles of the heart appears to be a complex process. Calcium can be conceived to interact with biomembranes through two processes which are energy independent and energy dependent. The energy independent mechanism reflects passive adsorption of calcium on the membranes due to the presence of phospholipids (92). On the other hand, the energy dependent mechanism requiring ATP is usually believed to be of two types, namely calcium binding and calcium uptake. Recently, calcium binding and uptake by cardiac microsomes have been found to be two separate processes which may initially share common sites on the membrane (93). Calcium binding has been considered to occur at an intracellular release site that initiates systole by delivering calcium to the contractile apparatus whereas calcium uptake has been suggested to represent the accumulation of calcium into an intracellular storage site whose calcium content indirectly determines the amount of calcium that is delivered to the contractile apparatus (47). It is likely that calcium adsorption, binding and uptake processes also occur in mitochondrial membranes, however, their role in myocardial function is not clearly apparent. Since the sarcolemmal preparation employed in this study did not show ATP dependent calcium binding or uptake (89), it is suggested that these processes may be absent in the cell membrane. It is also possible that mechanisms for ATP dependent calcium binding and uptake are inactivated during the isolation procedure. At any rate, binding and uptake processes by microsomes and mitochondria and calcium adsorption by these fractions as well as sarcolemma of the heart can be considered to participate in the regulation of intracellular calcium.

In this study quinidine has been demonstrated to depress calcium uptake by

cardiac heavy microsomes. This is in agreement with the findings of other investigators with both cardiac and skeletal muscle fragments of sarcoplasmic reticulum (4, 6, 7). Since calcium uptake as measured in this study is a result of active influx and passive efflux of calcium across the vesicular membranes, the observed decrease in calcium uptake due to quinidine may be a consequence of the action of this drug on these processes. This is substantiated by the fact that quinidine has been reported to decrease the activity of microsomal ATPase which is considered to be involved in the active transport of calcium (4, 7). Since the depressant effect of quinidine was not apparent at low concentrations of Mg^{++}, it is likely that quinidine may be acting on the breakdown of a phosphoprotein, an intermediate in the transport of calcium (94). This suggestion is further supported by a recently proposed mechanism of quinidine on the inhibition of the cardiac sarcotubule - γ - $AT^{32}P$ reaction (95). Although quinidine has been reported to be capable of releasing calcium from the microsomal fraction (8), convincing data is required to draw a firm conclusion in this regard.

The observed depression of calcium uptake by quinidine may not be due to changes in calcium binding ability of cardiac microsomes. This is borne by the fact that under conditions when calcium uptake was depressed, calcium binding was not altered. On the other hand, calcium binding was stimulated at initial stages of incubation or at low Mg^{++} concentrations. Quinidine has also been found to have a slight stimulatory effect on ATP dependent calcium binding by skeletal muscle sarcoplasmic reticulum (85). This stimulatory effect of quinidine on calcium binding appears to be masked at high concentrations of Mg^{++} under conditions when quinidine is inhibiting the calcium uptake site. In the absence of Mg^{++} and ATP calcium

adsorption by heavy microsomes was markedly stimulated. Thus it appears that quinidine has a complex mode of action on the microsomal membrane and Mg^{++} seems to play an important role in determining the locus of its action.

Unlike quinidine, procaine amide had no effect on microsomal calcium uptake while lidocaine showed an initial stimulatory effect. Shinebourne et al. (6) were unable to observe an effect of lidocaine on calcium uptake by cardiac microsomes. In this study lidocaine, like quinidine, did not influence calcium binding whereas procaine amide stimulated it. Furthermore, quinidine and procaine amide were found to stimulate microsomal calcium adsorption whereas lidocaine had a depressant effect. These results suggest that quinidine, procaine amide and lidocaine act on different sites involved in the process of calcium transport by the heart sarcoplasmic reticulum. Although these drugs have been shown to have varying degrees of cardiodepressant action (3) it seems unlikely that these agents exert their cardiac effects solely due to their influence on microsomal calcium transport.

It was demonstrated in this study that quinidine depressed mitochondrial calcium binding and uptake and stimulated mitochondrial calcium adsorption. Noack et al. (77) has also shown that quinidine decreased the rate of calcium uptake by heart mitochondria. The depressant effect of quinidine on calcium binding by mitochondria was found to be Mg^{++} and ATP dependent whereas calcium uptake was only Mg^{++} dependent. On the basis of the available information concerning mitochondrial calcium transport it is difficult to state with certainty the exact site of drug action and therefore further studies are clearly needed to elucidate this mechanism. However, it should be noted that the mechanism of quinidine action on

mitochondria seems to be different from lidocaine and procaine amide. For example, lidocaine, but not procaine amide, depressed calcium binding. Both procaine amide and lidocaine had no influence on mitochondrial calcium uptake. Procaine amide produced a biphasic effect on mitochondrial calcium adsorption whereas lidocaine depressed it. Lidocaine and procaine amide have also been reported to have no effect on heart mitochondria calcium uptake (77). The significance of these drugs on mitochondrial calcium transport with respect to their cardiodepressant actions is a matter of speculation at present.

We have found that quinidine stimulated sarcolemmal calcium adsorption. Since calcium is a well-known membrane stabilizer (92), it is possible that the cardiodepressant effect of quinidine may partly be mediated through its action on the sarcolemma. In contrast, quinidine has been shown to decrease calcium adsorption by skeletal muscle sarcolemma (5). This difference in the action of quinidine on the sarcolemma of cardiac and skeletal muscle may partly explain the difference in the effects of this drug on the mechanical properties of these muscle. The results concerning the effects of quinidine on calcium movements in atrial muscle and $Na^+ - K^+$ ATPase of the ventricular muscle can also be interpreted as to reflect the sarcolemmal site of quinidine action (86, 87). Since lidocaine, unlike quinidine, depressed sarcolemmal calcium adsorption whereas procaine amide was without effect, these results suggest differences in the mode of action of these agents on cardiac muscle.

In this study we have shown that quinidine affects calcium transport properties of sarcoplasmic reticulum, mitochondria and sarcolemma. The in vitro concentrations that were used to elicit these effects appear to be higher than the doses of this drug

which are employed therapeutically for the treatment of common arrhythmias. However, it is possible that the local concentration of this drug at the microsomal, mitochondrial and sarcolemmal sites may be different than that in the circulation. It should be noted that quinidine has been shown to bind with sarcoplasmic reticulum, mitochondria and sarcolemma (7, 84, 96). Thus it appears that the cardiodepressant effect at high doses of quinidine may be due to the modification of calcium transport properties of these organelles. This study does not in any way rule out the modification of other functions of these membrane systems by quinidine.

CONCLUSIONS

In this study the effects of quinidine, procaine amide and lidocaine on the calcium transport properties of heart sarcoplasmic reticulum, mitochondria and sarcolemma under various experimental conditions were investigated. From the data obtained in the study, the following conclusions are drawn:

a) Quinidine was found to inhibit calcium transport by heart mitochondria as well as sarcoplasmic reticulum.

b) The mode of quinidine action on calcium transport seems to be complex in nature in which Mg^{++} plays a crucial role in determining its effects.

c) In addition to its action on sarcoplasmic reticulum and mitochondria, quinidine has been demonstrated to influence sarcolemmal calcium adsorption.

d) The site of quinidine action on heart membranes appears to be different from other agents such as procaine amide and lidocaine.

e) The cardiodepressant effect at high doses of quinidine may be due to a modification of calcium transport properties of cell organelles but the data does not rule out the modification of the other functions of these membrane systems by quinidine.

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